

IN THE CLAIMS

Please amend the claims as follows:

Claim 1 (Currently Amended): A method for producing a heterologous RNA of interest, ~~which the method is characterized in that it comprises at least the following steps~~ comprising:

(1) transforming the mitochondria of yeast cells lacking mitochondrial DNA with a mitochondrial transcription vector ~~comprising that comprises~~ comprising at least one copy of the DNA encoding said heterologous RNA of interest under the control of regulatory element(s) for mitochondrial transcription, and a mitochondrial transformation reporter gene or a fragment of said reporter gene;

(2) identifying the yeast mitochondrial transformants that have incorporated the DNA of interest;

(3) culturing the yeast mitochondrial transformants selected in step (2);

(4) isolating the mitochondria from the yeast mitochondrial transformants obtained in ~~step~~ (3), and

(5) extracting and purifying the heterologous RNA of interest from said mitochondria.

Claim 2 (Currently Amended): The method as claimed in claim 1, ~~characterized in that~~ wherein said yeast cells lacking mitochondrial DNA are *rho*⁰ cells.

Claim 3 (Currently Amended): The method as claimed in claim 1, wherein ~~or claim 2, characterized in that~~ said cells lacking mitochondrial DNA are obtained from a Δ *SUV3* or Δ *DSS1* strain.

Claim 4 (Currently Amended): The method as claimed in ~~any one of claims 1 to 3,~~
~~characterized in that~~ claim 1, wherein said cells lacking mitochondrial DNA comprise a
chromosomal copy of a gene encoding an exogenous RNA polymerase and including
includes a mitochondrial targeting signal.

Claim 5 (Currently Amended): The method as claimed in ~~any one of claims 1 to 4,~~
~~characterized in that~~ claim 1, wherein said DNA encoding the RNA of interest is under the
control of a promoter and a transcription terminator that are functional in yeast mitochondria.

Claim 6 (Currently Amended): The method as claimed in ~~any one of claims 1 to 5,~~
~~characterized in that~~ claim 1, wherein said mitochondrial transformation reporter gene is a
gene encoding one of the proteins of a yeast respiratory chain.

Claim 7 (Currently Amended): The method as claimed in ~~any one of claims 1 to 6,~~
~~characterized in that~~ claim 1, wherein said mitochondrial transcription vector comprises the
sequence of an origin of replication of the mitochondrial DNA.

Claim 8 (Currently Amended): The method as claimed in ~~any one of claims 1 to 7,~~
~~characterized in that~~ claim 1, wherein the transformation according to ~~step~~ (1) comprises the
adsorption of said mitochondrial transcription vector onto metal microprojectiles and the
projection of said microprojectiles onto said cells.

Claim 9 (Currently Amended): The method as claimed in ~~any one of claims 1 to 8,~~
~~characterized in that~~ claim 1, wherein ~~step~~ (1) comprises the cotransformation of said yeast

cells with said mitochondrial transcription vector and a vector that is replicative in yeast, comprising a nuclear selection marker.

Claim 10 (Currently Amended): The method as claimed in claim 9, ~~characterized in that wherein~~ said nuclear marker is an auxotrophic marker of said transformed cells.

Claim 11 (Currently Amended): The method as claimed in ~~any one of claims 1 to 10, characterized in that step~~ claim 1, wherein (2) comprises:

(a₀) crossing the yeast mitochondrial transformants obtained in step (1) with a yeast tester strain of *rho*⁺ mit⁻ type,

(b₀) identifying the mitochondrial transformants which, once crossed, give diploid cells capable of growing on a non-fermentable medium, and

(c₀) repeating said crossing until isolated yeast colonies identified as being mitochondrial transformants carrying the mitochondrial transformation vector are obtained.

Claim 12 (Currently Amended): The method as claimed in claim 9, ~~wherein or claim 10, characterized in that step~~ (2) comprises:

(a₁) a first selection or preselection of the yeast cells by means of said nuclear marker, by culturing in an appropriate medium, and

(b₁) a second selection from the yeast cells selected in (a₁), in accordance with steps (a₀), (b₀) and (c₀), as defined in claim 11.

Claim 13 (Currently Amended): The method as claimed in ~~any one of claims 1 to 12, characterized in that~~ claim 1, wherein the isolation of the mitochondria, in accordance with ~~step~~ (4) of the method, comprises lysis or grinding of said cells, and then at least two

centrifugation steps, at speeds preferably of between 750 g and 12,500 g, and recovery of the final centrifugation pellet.

Claim 14 (Currently Amended): The method as claimed in ~~any one of claims 1 to 13,~~
~~characterized in that~~ claim 1, wherein step (5) advantageously comprises:

- eliminating the contaminating nucleic acids in the presence of appropriate buffers, the first buffer comprising at least one divalent ion-chelating agent, and the second buffer comprising an RNase and, optionally, a DNase,
- lysing the mitochondria in the presence of at least one detergent and a divalent ion-chelating agent and within a pH range of between 7 and 8, and
- isolating and purifying the RNA of interest.

Claim 15 (Currently Amended): A modified yeast cell, ~~characterized in that it~~ which lacks mitochondrial DNA and ~~in that it comprises~~ that comprises a chromosomal copy of a gene encoding an exogenous RNA polymerase and including a mitochondrial targeting signal.

Claim 16 (Currently Amended): A modified yeast cell, ~~characterized in that it~~ which lacks mitochondrial DNA and ~~in that it's~~ the mitochondria are transformed with a mitochondrial transcription vector as defined in ~~claims 1 and 5 to 7~~ claim 1.

Claim 17 (Currently Amended): The modified yeast cell ~~as claimed in~~ of claim 15, ~~which or claim 16, characterized in that it~~ is obtained from a $\Delta SUV3$ or $\Delta DSS1$ strain.

Claim 18 (Currently Amended): The modified yeast cell ~~as claimed in any one of claims 15 to 17, characterized in that it~~ of claim 15, which is obtained from a ρ^0 strain.

Claim 19 (Canceled).

Claim 20 (Currently Amended): A system for carrying out the industrial production of a heterologous RNA of interest, ~~characterized in that it comprises~~ the system comprising:

- yeast cells lacking mitochondrial DNA, ~~in particular of ρ^0 strain,~~ transformed with at least one mitochondrial transcription vector as defined in ~~claims 1 to 5 and 7~~ claim 1,
- at least one suitable culture medium for selecting said transformed cells,
- yeast tester cells of ρ^+ mit⁻ type,
- appropriate fermenters and culture media, and
- appropriate reagents for isolating the mitochondria from synthetic ρ^- cells and extracting the heterologous RNA of interest ~~therefrom~~.